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Complement

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The term "complement" is used to include a complex group of interacting blood proteins and glycoproteins found in all vertebrates. These proteins have as their primary functions the production and regulation of inflammation, the opsonization of foreign materials for phagocytosis, and the mediation of direct cytotoxicity against various cells and microorganisms. The first evidence for the existence of such a system arose in the late 19th century during studies of the mechanism of host defense against invading bacteria and studies of the mechanism of destruction of foreign or mismatched transfused cells (1–3). These studies demonstrated that individuals are capable of responding to invading microorganisms or to injected foreign cells by the production of antibody. Such antibodies are able *in vitro* to agglutinate the organisms or foreign cells used in the immunization but are unable to mediate cell death. It was discovered that the addition of fresh serum to a mixture containing specific antibody and the microorganism or immunizing cell often led to cell death. The importance of this property of fresh serum was quickly recognized, and a series of investigations was begun to define the biochemical and biologic basis of the phenomenon of cell lysis.

Work over the next several decades demonstrated the

complex nature of the lysis phenomenon. It quickly became clear that the lytic material, present in all fresh sera, was not a simple substance but could be separated into several principles by even the crude chemical techniques then available. For example, dialysis of the serum against water separated the lytic material into a precipitable euglobulin fraction and a soluble pseudoglobulin fraction. Neither fraction had lytic activity when tested, but mixing the fractions restored this activity. The materials that acted together to produce this cytotoxic response were collectively termed alexin by Bordet, and first "addiment," and later complement by Ehrlich. Although some early workers doubted that the reactions leading to lysis followed simple chemical rules, it was soon recognized that this was indeed the case and experimental systems were established to permit detailed study of these biochemical events. Many of the experimental models established at the turn of the century are still in use today and it is valuable to consider a few of them in some detail.

Much of the early work was directed toward establishing an *in vitro* system designed to allow for analysis of each of the steps involved in complement-mediated cell death. Erythrocytes from many species were screened to determine which were the most easily lysed by antibody and complement. Sheep erythrocytes proved to be particularly useful since, when sensitized with antibody, they were highly susceptible to the lytic action of complement. It was also discovered that sheep erythrocytes have on their surface a potent lipopolysaccharide antigen (termed Forssman antigen) (4) and that high titered antibody to this antigen could be prepared conveniently in the rabbit. In general, sera from all mammals could be used as a complement source, but the degree of lysis varied greatly among different species. It was found that fresh guinea pig serum was the most potent lytic serum easily available. A test system utilizing sheep erythrocytes sensitized with rabbit antibody for studies of lysis in fresh guinea pig serum was commonly employed. With time, it became possible to create specific intermediates bearing various complement components on the surface of the antibody-coated sheep erythrocytes and to study the interaction of each newly defined complement protein with the appropriate cellular intermediate in the complement sequence. Early efforts focused almost exclusively on the events that occur in the lysis of the antibody-coated sheep red cell. Such events now comprise the classical complement pathway. In more recent years it has become clear that another closely related series of proteins is often involved in the lysis of bacteria with or without the intervention of antibody. These bacteriolytic proteins make up the alternative complement pathway. The two pathways of activation converge at the step of C3 activation and engage the later lytic components in the complement cascade (Fig. 1). The classical pathway, in general, is initiated by the formation of an antigen–antibody complex. Recognition of the antigen–antibody complex by the proteins of the classical pathway leads to sequential formation of enzymes with serine protease activity. These cleave and activate C3. The proteins of the alternative pathway mediate this same end result, albeit with slower kinetics of activation. Cleaved C3 (C3b) interacts with the C3-cleaving enzymes of either the classical or alternative pathways and alters their substrate specificities such that they are able to cleave C5. Cleaved C5 (C5b) then interacts with the remaining numbered components, C6, C7, C8, and C9, and these five terminal complement components, acting in concert, mediate cell lysis. This general scheme of complement activation is presented in Fig. 1.

The complement system is essentially entirely conserved throughout the mammalian species that have been studied, and the proteins are often (but not always) interchangeable across species. The system is by far best characterized in the human and guinea pig, although much data on the murine proteins have evolved recently through molecular techniques.

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The nine proteins of the classical pathway are designated by an uppercase letter C followed by a number. The numbers generally follow the order of action of the components, with the exception of C4, which acts before C2 and C3. Components acting solely in the alternative pathway are designated by letters. Regulatory proteins are designated by a descriptive title (e.g., C4 binding protein) or, in the case of those proteins closely associated with the alternative pathway, a letter (e.g., factor H). Single components or multicomponent complexes that have enzymatic activity are designated by a bar over the component(s) in question (e.g., $\overline{C1r_2}$). Molecules that have lost activity through chemical denaturation or by the action of a control protein are usually designated by a prefix lower case i (e.g., iC3). Fragments or subunits of the various components are designated by a lowercase letter suffix (e.g., C3b).

THE CLASSICAL COMPLEMENT PATHWAY

The Role of Immunoglobulin

Activation of the classical pathway is initiated by the binding of C1, the first component in the cascade, to an antigen–antibody complex and the subsequent activation of the antibody-bound C1 (5,6). The steps have been examined in considerable detail. Not all classes of antibody are capable of binding C1 to initiate the classical pathway. IgG and IgM antibodies have this ability, but IgE, IgD, and IgA antibodies do not (5). Studies in a number of test systems have demonstrated that a single molecule of IgM bound to a particulate antigen is capable of binding one molecule of C1, a complex zymogen protease (7). However, the processes of antibody binding of C1 and the activation of C1 to a protease capable of cleaving C4 and C2 are not equivalent (6). To mediate C1 activation, the IgM antibody molecule must engage the antigen by more than one of its Fab arms. This was inferred from studies of the binding and activation of C1 on anti-hapten IgM sensitized sheep erythrocytes coated with various den-

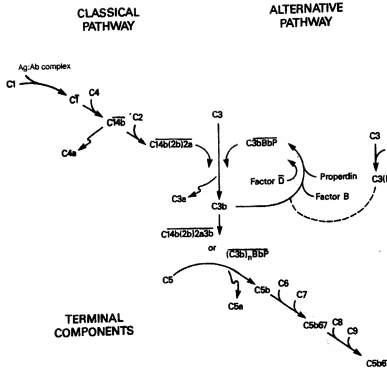

FIG. 1. A schematic diagram of complement activation. Regulatory proteins, side reactions, and inactive fragments have been omitted for clarity. Enzymatically active species are designated by *overbars*.

sities of a defined hapten, and by analysis of C1 activation by idiotype anti-idiotype immune complexes containing IgM (8,9). The finding that multiple variable region sites must be bound to an antigenic surface for C1 activation suggests that binding facilitates a conformational change in the antibody that promotes C1 activation.

On the other hand, in most of the model systems studied, two IgG molecules side by side (a doublet) are required for C1 binding and activation (10). In molecular terms, the requirement for an IgG doublet greatly reduces the efficiency of IgG as compared to IgM in inducing classical pathway activation. In most systems that examine the lysis of target particles, hundreds or thousands of IgG molecules must be supplied before, by chance, two molecules come to lie sufficiently close together on a surface to produce a doublet. If the distribution of antigen molecules on a surface precludes the possibility of two IgG molecules coming to lie sufficiently close together, the IgG may not activate complement at all. In fact, it has been shown that IgA, under some circumstances, blocks complement activation by IgG antibody by inhibiting the formation of doublets (11). Not all IgG subclasses are capable of activating the classical pathway. Human IgG1, IgG2, and IgG3 are all activators of the classical pathway, but IgG4 is not. In mouse systems, γ2a, γ2b, and γ3 activate the classical complement pathway, and in guinea pig, γ2 antibodies activate the classical pathway.

It is not known whether the binding of IgG antibody to its substrate causes a structural change in the antibody that induces increased affinity for C1. Binding of C1 to surface-bound IgG doublets may simply be a consequence of cooperative binding effects. It has been shown that IgG monomer will interact with native C1, albeit weakly (12). A cluster of closely associated IgG molecules may allow for multiple points of C1 attachment, thus stabilizing the IgG–C1 complex. Binding of C1 proceeds via its attachment to the CH_2 domain of the Fc portion of the IgG molecule (13,14).

Recent data suggest that successful activation of C1 requires multiple points of contact between C1 and the activator. In the case of IgG it has been suggested that not only must the CH_2 domain bind C1 but there must be a second point of contact with the CH_1 domain for activation to occur (15). In keeping with this, it is also suggested that a rather narrow range of angles between the Fab arms of IgG are optimal for C1 activation (8). Accordingly, both simple spatial clustering and structural rearrangements induced by antigen binding may have a role in C1 activation by IgG. Recently, a C1-binding motif consisting of a trio of charged amino acid residues has been identified in the CH_2 domain of murine IgG. These residues are highly conserved in most mammalian IgGs examined and appear to be necessary, but not sufficient, for efficient C1 binding (16).

C1

C1 exists in serum as a three-subunit macromolecule with the subunits held together in the presence of ionic calcium (7,17). C1q is the subunit that binds to an antigen–antibody complex via the antibody CH_2 domain; it has a molecular weight (MW) of about 400,000 and is composed of 18 chains: three chain types termed A, B, and C with six copies of each per molecule. The protein has a central

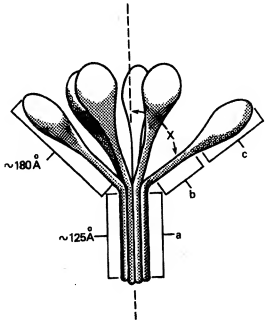

FIG. 2. A conceptualization of C1q. The central core is designated by (a) and the collagen-like fibrillar arms by (b). The globular heads which bind to immunoglobulins are designated by (c). Angle X ranges from 20° to 80°.

core and six radiating arms, each of which ends in a pod-like globular structure (Fig. 2). The amino terminal end of each arm has a triple-helix, collagen-like structure and can be cleaved by bacterial collagenases. These regions are rich in glycine, hydroxylysine, and hydroxyproline. Binding of C1q to the CH_2 domain of the antibody occurs through the podlike carboxyl terminus at the end of each arm. Potentially, each of the six C1q arms can bind to one CH_2 domain; it is assumed that multiple C1q–antibody interactions are required for firm binding (see previous discussion). C1q is found in serum in association with two additional C1 subunits: C1r and C1s. C1r and C1s both have molecular weights of about 85,000 and are single-chain proenzymatic forms of serine proteases. Current models envision the two C1r molecules and the two C1s molecules arranged linearly with the two C1r molecules in contact in the center of the claim and the two C1s molecules at the ends (18). When C1 is incubated in ethylene diamine tetraacetate (EDTA)-containing buffers to remove calcium, the molecule dissociates. The two chains of C1r molecules remain associated and the two C1s chains are free in solution. C1q remains intact but loses its C1r and C1s.

The $C1r_2$–$C1s_2$ tetramer is believed to associate with the collagenous regions of the C1q molecule when calcium is present (18). Following binding of the intact macromolecular zymogen form of C1 to an antigen–antibody complex, the C1 undergoes enzymatic activation to become an active serine protease. Activation is associated with cleavage of each of the C1r chains and each of the C1s chains into a heavy chain of 57,000 and light chain of 28,000 daltons. The enzymatic sites reside in each of the smaller subunits in both cases (19).

In *in vitro* studies, activated $\overline{C1r}$ is capable of cleaving and activating C1s; activated C1s has broader enzymatic specificity and is the enzyme responsible for the cleavage of both C4 and C2. The mechanism by which the binding of C1q leads to activation of C1r and C1s is unknown. Some have suggested that this involves an intramolecular rearrangement within the intact C1 molecule. The mechanism of $\overline{C1r}$ cleavage of C1s is also unknown. Although some investigators have suggested that activated $\overline{C1r}$ cleaves the C1s chains within a single macromolecular C1 molecule, others have hypothesized that C1r cleavage of C1s is actually an intermolecular event requiring the proper alignment of two C1 molecules on an activating surface.

C4

The binding and activation of C1 leads to the generation of an enzyme capable of coordinating with and cleaving the second protein in the cascade, C4. C4 is composed of three disulfide-linked chains termed α, β, and γ with MWs of 93,000, 78,000, and 33,000, respectively. The protein is synthesized as a single-chain precursor (proC4) and the three-chain structure is formed as a post-translational event (20,21).

On interaction with $\overline{C1s}$, the C4 α chain is cleaved with release of a small fragment, C4a (9,000 daltons), from its amino terminus. C4a is discussed in detail in the section on anaphylatoxins. The larger fragment, C4b, contains the modified α chain (α′), β, and γ and continues the complement cascade. The binding of C4b to a surface, unlike the binding of C1, proceeds via formation of a covalent ester or amide bond and is apparently highly analogous to C3b binding (see later discussion) (22). An antibody site with a bound, active $\overline{C1}$ will cleave multiple C4 molecules, and a cluster of C4 molecules will bind to the region surrounding the antibody–$\overline{C1}$ site. This represents an amplification step in classical pathway activation since a single C1-fixing site leads to the activation of multiple C4 molecules. Not all deposited C4 molecules are equally active hemolytically (23). Those which bind to, or close to, the antibody–$\overline{C1}$ complex will continue the complement cascade. Bound C4b appears to protect adjacent $\overline{C1}$ molecules from the action of C1 inhibitor (24). This serves to promote complement activation at the site of an immune complex and limits the effect of nonspecific activation of C1. The binding of C4b to certain targets may have an effect on biologic activity. For example, certain viruses may be neutralized by the deposition of multiple C4 molecules on their surface, preventing their binding to a suitable host cell (25).

C2

The third protein in the antigen–antibody recognition steps of the classical pathway is C2. This molecule con-

sists of a single peptide chain of 95,000 daltons. In the presence of Mg^{2+} ion, C2 binds to C4b and is cleaved by adjacent $\overline{C1s}$. Two fragments are formed. A small fragment (C2b, MW 30,000) is cleaved from the molecule, and the larger fragment $\overline{C2a}$ remains associated with C4b to continue the cascade (26). The complex of $\overline{C2a}$ and C4b is endowed with new enzymatic activity: the ability to coordinate with and to cleave C3. The active enzymatic site, again a serine protease, resides on the $\overline{C2a}$ portion of the molecule. Some data suggest that the C2b remains as part of the complex, acting as the C4b-binding site (27). The C4b in the complex binds the C3 molecule and makes it accessible to $\overline{C2a}$ cleavage. The $\overline{C4b2a}$ complex, termed the classical pathway C3 convertase, is labile and undergoes decay with physical release of the C2a as an enzymatically inactive fragment. The C4b left behind on the antigenic surface can bind another C2 molecule, which on cleavage by $\overline{C1}$ will again form the C3 convertase enzyme.

REGULATION OF THE CLASSICAL PATHWAY

C1 Inhibitor

The C1 inhibitor (C1INH) is a single-chain serum glycoprotein of 105,000 daltons with an unusually high carbohydrate content (about 35 to 40%) (28). The protein functions by combining in 1:1 stoichiometry with the active site on each activated $\overline{C1r}$ and $\overline{C1s}$ to destroy its protease activity. Since there are two $\overline{C1r}$ protease sites and two $\overline{C1s}$ protease sites per $\overline{C1}$ molecule, one molecule of activated C1 can, in theory, react with four molecules of C1–INH. Binding of C1–INH to activated $\overline{C1}$ induces disassembly of the C1 molecule with release of two molecules of a $\overline{C1r}\overline{C1s}$ $(C1INH)_2$ complex (29,30). The C1q is presumably left behind on the activating surface where it may interact with additional plasma C1r and C1s or with specific C1q receptors on a variety of cells (see section on receptors). C1–INH chemistry has been examined in considerable detail. It is a member of the family of proteins termed serpins (serine protease inhibitors) and acts by presenting a bait sequence to the enzyme to be inhibited. In the case of the C1–INH, this bait sequence contains a critical arginine at position 444 (31). The enzyme to be inhibited cleaves the C1–INH at the active-site arginine into two fragments of 96,000 and 9,000 MW. Upon cleavage, a reactive site is exposed in the 96,000-dalton fragment which binds to the active site on the enzyme to be inhibited, forming a stable complex that is resistant to boiling in SDS (28). C1–INH is also reported to interact with C1 before its activation. This interaction appears to inhibit C1 activation by spontaneous autocatalysis or nonimmune activators but not by antigen–antibody complexes (32). Interestingly, binding of C1–INH to $\overline{C1r}$ leads to loss of detectable C1r antigen using most anti-C1r antisera. Thus disappearance of antigenically detectable C1r, along with the appearance of cleaved C1r and C1s chains on SDS–polyacrylamide gel electrophoresis (SDS–PAGE), allows one to follow the kinetics of C1 activation and inhibition (33).

C4b Regulation and C4-Binding Protein

The activity of C4b is under the control of a series of membrane-bound and fluid-phase proteins. In this section we describe interactions that can occur in the fluid phase. A separate section discusses all the membrane-bound complement regulators.

C4-binding protein (C4BP) is an interesting molecule of approximately 570,000 daltons. The molecule consists of seven identical chains that are associated by disulfide bonds at their carboxy termini, forming a central "core" (34). The amino terminal portions of the seven chains form tentacular arms which radiate from the core and bear the C4b binding domains, which are homologous to the binding domains of a broad family of proteins that interact with C3b and C4b (see later discussion). C4BP binds to C4b (not C4) and exerts two distinct regulatory actions. The intrinsic dissociation rate of the C4b2a complex is increased, thus shortening the survival of any given classical C3 convertase enzyme (35). In addition, C4b bound by C4BP becomes susceptible to proteolysis by factor I, also known as C3b/C4b inactivator. Factor I cleaves the α' chain of C4b in two places, releasing a four-chain complex termed C4c and leaving a small portion, C4d, covalently bound to the original acceptor surface (36). C4d can no longer support complement convertase activity. Factor I cleavage of C4b on surfaces can proceed without C4BP, albeit slowly, but C4BP is requisite in the fluid phase.

THE ALTERNATIVE PATHWAY OF COMPLEMENT ACTIVATION

History

An alternative pathway for complement activation was described in 1954 by Louis Pillemer and his co-workers at Case Western Reserve University during their investigations of the inactivation of C3 and late-acting complement components by yeast cell walls. They demonstrated that an insoluble yeast cell wall preparation, zymosan, could completely consume C3 during a 37°C incubation in serum without affecting C1, C4, or C2 titers (37). This inactivation had the characteristics of an enzymatic reaction and required factors that could be removed from serum by preincubation with zymosan at 17°C. These factors differed from antibodies in that their absorption from serum required magnesium ions, temperatures above 10°C, a pH of 6.5 to 8.2, and low ionic strength. This led to the suggestion that consumption of C3 occurred by enzymatic activation via a new pathway which was termed the properdin system. Moreover, it was demonstrated that this system played an important role in the serum bactericidal reaction, in viral neutralization, and in the acid lysis of erythrocytes from patients with paroxysmal nocturnal hemoglobinuria. Unfortunately, subsequent recognition that natural antibody to zymosan is present in normal serum led to the belief that Pillemer's data reflected only the activation of the classical pathway

by preexisting antibody (38). Thus the fundamental discoveries of Pillemer and his colleagues were largely ignored until the alternative pathway was "rediscovered" a decade later.

There are six normal serum proteins thought to be important in the initiation and control of alternative pathway activation. These are factor B, factor D, properdin, factor H (β1H), factor I (C3b/C4b inactivator), and C3 itself (39).

Factor D

Factor D is a 25,000-dalton, single-chain glycoprotein, with γ-globulin electrophoretic mobility when purified but α-globulin electrophoretic mobility in serum (40). It is a trace protein in serum (1 to 2 μg/ml), and its low concentration may make it rate limiting in assembly of the alternative pathway C3 convertase, C3bBb. No evidence of cleavage of factor D has been found and it is presumed to circulate in the active state. It has sequence homology with other serine proteases but does not hydrolyze synthetic esters and is relatively resistant to the archetypal serine protease inhibitor diisopropylfluorophosphate (DFP) (41). It has no activity on its natural substrate, factor B, until the latter is bound by C3b. Thus it is most likely that factor D is able to express its proteolytic activity only after exposure of a cryptic site on factor B by the C3b interaction.

Factor B

Factor B is a single-chain, 93,000-dalton, β-globulin zymogen serine protease. In the presence of Mg^{2+}, factor B forms a stoichiometric complex with C3b with a molar ratio of 1:1 (42). On cleavage by factor D, two fragments are formed. The smaller 30,000-dalton fragment, Ba, is released, while the larger 63,000-dalton fragment, $\overline{Bb}$, remains associated with C3b. The latter fragment contains the protease activity (43). The complex $\overline{C3bBb}$ is termed the alternative pathway C3 convertase. This complex is quite labile and $\overline{Bb}$ dissociates spontaneously from C3b under physiologic conditions. Ba and Bb are reported to have opposing regulatory effects on B lymphocyte function and Bb is reported to augment the spreading of phagocytic cells on a surface.

Properdin

Properdin (from the Latin *perdere*, to destroy), the protein through which the alternative pathway was discovered, is a γ-globulin of approximately 143,000 to 156,000 daltons, consisting of three apparently identical subunits held together noncovalently (44). Its serum concentration is about 25 μg/ml. There are two forms of properdin, native (nP) and activated (P), which apparently differ from each other only by a small conformational change (45). Native properdin can bind to the assembled alternative pathway C3 convertase ($\overline{C3bBb}$) but cannot bind to C3b alone. Its function in this circumstance is to reduce the rate of decay of the convertase and thus promote alternative pathway activation. Activated properdin can, in addition, bind to C3b on particles or in the fluid phase in the absence of factor B and then promote the assembly of $\overline{C3bBb}$. Factors regulating the conversion of nP to P are largely unknown, and some authors have questioned whether two distinct forms truly exist. Spontaneous conversion of nP to P in purified protein preparations has been observed to occur, and P is the form of the molecule eluted from alternative pathway activators after incubation in serum. On the other hand, P does not revert to nP spontaneously but is reported to do so after incubation with the denaturant guanidine.

Factor H

Factor H (formerly known as β1H) is a 150,000-dalton, single-chain peptide of β electrophoretic mobility. Its serum concentration is about 500 μg/ml (46). Gel filtration studies indicate that factor H may circulate as a dimer under physiologic conditions. Unlike the previously described proteins, factors B and D and properdin, which are important in the assembly of the alternative pathway C3 convertase, factor H functions to downregulate C3-cleaving convertase activity. This is accomplished by competition with both B and $\overline{Bb}$ for C3b binding, thereby inhibiting convertase formation and accelerating decay of existing convertase complexes (47). Factor H is also a necessary cofactor for the inactivation of C3b by factor I (48). The binding of H to C3b does not require cations but is enhanced by low ionic strength. Factor H can bind to C3b on surfaces or in the fluid phase, although with varying affinities depending on the chemical nature of the environment in which the C3b is found. As explained later, this varying affinity of H for C3b may be the most important determinant of whether or not a particle will activate the alternative pathway.

Factor I (C3b/C4b Inactivator)

Factor I is a 90,000-dalton, β-globulin glycoprotein composed of two chains of 50,000 and 40,000 daltons held together by disulfide bonds (49). It demonstrates proteolytic activity toward two substrate molecules: C3b and C4b. In the presence of the appropriate cofactors, factor I effects two sequential cleavages of C4b and three cleavages of C3b (50). These result in defined breakdown products with altered activity (see later discussion). The active site is on the 40,000-dalton light chain and is not inhibitable by DFP, soybean trypsin inhibitor, tosyl-L-lysine chloromethyl ketone (TLCK), benzamidine, or phenylmethyl sulfonyl fluoride (PMSF). Nonetheless, sequence analysis has demonstrated a high degree of homology between factor I and known serine proteases. Thus the activity of this enzyme is probably controlled by a requirement for configurational changes in its substrates (C3b or

C4b) mediated by essential cofactors (factor H or C4-binding protein), before exposure of the site for enzymatic cleavage. This is analogous to the mechanism of enzymatic activity of factor D on factor B.

C3

C3, because of its central role in both classical and alternative pathway activation and because of its major role in the host defense process, has been the focal point of much research on complement activation. Human C3 is a 195,000-dalton glycoprotein of β electrophoretic mobility. It has two chains, one of 120,000 daltons (α chain) and the other of 75,000 daltons (β-chain) with carbohydrate present on both chains (51). Serum concentration is 1 to 2 mg/ml. C3, like C4, is synthesized as a single-chain precursor molecule (proC3) in which the β chain occupies the amino terminus. Excision of a series of four arginine residues yields the mature molecule.

Within the α chain resides an unusual thioester bond between a cysteinyl residue and a glutamic acid residue, separated by two intervening amino acids. This unstable bond, thought to be buried in a hydrophobic pocket, is responsible for the covalent binding reactions of C3 (as well as C4 and α_2-macroglobulin, which share this feature) (52).

When C3 is cleaved by either the classical pathway C3 convertase ($\overline{C4b2a}$) or the alternative pathway C3 convertase ($\overline{C3bBb}$), the α chain is divided into two unequal fragments, the larger of which remains covalently linked via disulfide bonds to the β chain. This molecule, designated C3b, has a MW of 185,000 daltons (53). The smaller fragment, C3a, represents the amino terminal 77 amino acids of the α chain and has diverse effects on cells with receptors for this peptide, including lymphocytes, mast cells, and endothelial cells (see later discussion). On cleavage of C3 to C3b, the molecule undergoes a complex rearrangement of tertiary structure, which exposes the internal thioester bond in the α chain (52,54). On exposure, the thioester bond can be broken by reaction with appropriate aldehydes, carboxyl groups, nitrogen nucleophiles, or by water itself. The result is a new covalent bond between C3b and an electron-donating group. On particles such as erythrocytes or zymosan, the formation of ester bonds seems to be favored (55), whereas proteinaceous immune complexes form amide, as well as ester, bonds with C3b. Since water can hydrolyze the thioester and is generally present in vast molar excess over all other potential electron donors, the process of C3b binding to surfaces or soluble immune complexes is inefficient. In general, many molecules of C3b will incorporate H_2O into the reactive bond for each molecule that is able to bond covalently to the complement-activating surface. Nonetheless, recent experiments in which C3 was cleaved to C3b in the presence of small molecules, such as amino acids and simple sugars, have demonstrated that some of these are preferential acceptors of the C3b molecule. In particular, glycerol, threonine, and raffinose are highly efficient at forming covalent bonds with C3b even in the presence of a large excess of H_2O (56). Immunoglobulin G also appears to be an efficient C3 acceptor (see later discussion).

C3b, after formation by either the classical or alternative pathway C3 convertase, is susceptible to further cleavage, which proceeds in several steps (see Fig. 3) (57,58). The first cleavage is made by factor I with H as an essential cofactor for fluid-phase cleavage and an accelerator for cleavage on surfaces. A second factor I-mediated α-chain cleavage occurs in rapid succession with the release of a 3,000-dalton fragment. The resultant three-chain molecule is termed iC3b. It can no longer function in the alternative pathway C3 convertase (or the C5 convertase of either pathway). The iC3b molecule has 68,000- and 43,000-dalton chains derived from the α′ chain of C3b and the unchanged 75,000-dalton β chain of the original molecule. Surface-bound iC3b undergoes a third cleavage at a site within the 68,000-dalton chain, which releases a three-chain, 145,000-dalton molecule termed C3c and leaves a 41,000-dalton fragment termed C3dg bound to the surface. This cleavage is carried out by factor I, but factor H does not appear to be a physiologically relevant cofactor (58). Instead the cell membrane C3b receptor, called CR1, appears to serve as cofactor for this step (see later discussion). A variety of enzymes, including trypsin, plasmin, and neutrophil elastase, can remove a 10,000-dalton amino terminal portion from C3dg to yield C3d. These same enzymes can generate C3d directly from iC3b. The breakdown of iC3b in the fluid phase appears to follow the same steps but proceeds very much more slowly and is less well characterized.

ALTERNATIVE PATHWAY ACTIVATION AND CONTROL

When C3b is generated in the course of classical pathway activation, it may serve as an initiator of the alternative pathway by providing a binding site for factor B. The binding requires Mg^{2+}. Binding to C3b is presumed to expose a cryptic cleavage site in the factor B molecule, which is the substrate for the protease, factor D. Ba is released, and the resulting bimolecular complex, $\overline{C3bBb}$, acts as the C3 convertase of the alternative pathway (59). The active enzymatic site is on Bb, but C3b is essential. C3b in the alternate pathway convertase is able to bind additional native C3 and in so doing makes the cleavage site in the C3 molecule available to the enzymatic activity of Bb. In this way, C3b in the alternative pathway C3 convertase is analogous to C4b of the classical pathway C3 convertase and $\overline{Bb}$ is analogous to $\overline{C2a}$. Factor D is not incorporated into the C3 convertase and may be reutilized. Enzymatic activity is controlled by several mechanisms. Factor H may displace factor $\overline{Bb}$ from the alternative pathway C3 convertase and also functions as a cofactor for factor I cleavage of C3b, terminating its ability to function in the convertase (60). In addition, the complex enzyme $\overline{C3bBb}$ has a tendency to dissociate even in the absence of regulatory proteins, with a $T_{1/2}$ of about 5 min at 30°C. Properdin counterbalances these controls

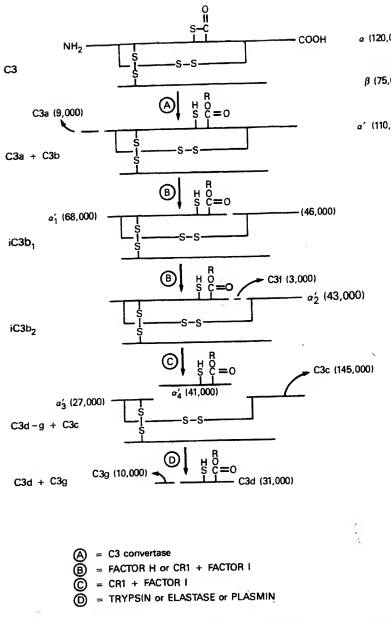

FIG. 3. C3 cleavage and the subsequent stepwise breakdown of C3b. Fragment molecular weights and enzymes responsible for the various cleavages are designated. Recent studies suggest that gp45-70 (membrane cofactor protein) is also a relevant cofactor for step B.

by decreasing the rate of the dissociation of C3bBb and therefore stabilizes enzymatic activity (59). Factors H and I are absolutely required to prevent spontaneous fluid-phase assembly of the alternative pathway C3 convertase and the subsequent consumption of alternative pathway components through unregulated activity of factor D and $\overline{C3bBb}$. This has been shown *in vitro* using the purified proteins of the alternative pathway and has also been confirmed *in vivo*, since patients genetically deficient in factor I or H have very low C3 levels and circulating C3 fragments (61). When additional C3b is formed by the action of $\overline{C3bBb}$, some portion of the new C3b may join the initial complex to form $\overline{(C3b)_2Bb}$. The second C3b provides a binding site for C5 and allows the new complex enzyme to function as the alternative pathway C5 convertase. Kinoshita et al. have very recently demonstrated that the second C3b responsible for C5-binding activity is actually covalently attached to the initial membrane-bound C3b residue.

Whenever C3b is created by classical complement pathway activation, it may serve as the nidus for formation of an alternative pathway C3 convertase. The alternative pathway convertase can deposit more C3b on the target surface, which can in turn form another alternative pathway convertase site. In this situation, the alternative pathway serves as an amplification mechanism for complement activation and is often referred to as the "amplification pathway."

The initial event of alternative pathway activation in the absence of classic pathway activation is less clear. The most likely sequence of events has been outlined by Pangburn and Muller-Eberhard (59,62). They showed that

the thioester bond in native C3 is not completely stable but subject to slow hydrolysis even in the absence of cleavage of the C3 molecule to C3a and C3b. C3 bearing a hydrolyzed thioester is termed $C3(H_2O)$ and has a number of properties of C3b, including the ability to initiate the alternative pathway by interacting with factor B, the ability to interact with factors H and I, and the capacity to serve as a ligand for cellular C3b receptors (63). Spontaneous hydrolysis of C3 with formation of an alternative pathway C3 convertase leads to a continuous low-level cleavage of C3 in serum called C3 "tickover." This baseline C3 turnover occurs at a slow rate because it is controlled by factors H and I. In the presence of alternative pathway-activating surfaces, such as rabbit erythrocytes, zymosan, or lipopolysaccharide, the tickover process is thought to provide the initial C3b, which binds to these surfaces to begin formation of the surface-bound alternative pathway C3 convertase.

Clearly, not all surfaces support the assembly of an alternative pathway C3 convertase whenever C3b by chance is bound to them through serum C3 tickover. This implies that inherent in the activation of the alternative pathway is the ability to discriminate among surfaces to which C3b binds. A further implication, since normal self-antigens do not activate the alternative pathway, is that the alternative pathway represents a primitive mechanism for distinguishing self-antigens from foreign antigens, such as yeast cell walls (zymosan) or bacterial cell walls or lipopolysaccharide. The molecular nature of this discriminatory ability is partially understood. Activators of the alternative pathway provide a "protected site" for C3b binding, that is, one in which the net effect is to favor factor B binding to C3b over factor H binding (59). In this setting, the alternative pathway C3 convertase will form and be able to cleave more C3 without the usual inhibition by factor H. With rare exception, the property of alternative pathway-activating potential correlates with a reduced affinity of surface-bound C3b for factor H. This has best been demonstrated in investigations of the role of cell surface sialic acids in control of alternative pathway activation (64,65). Although sheep erythrocytes do not activate the human alternative pathway, sheep cells from which sialic acid residues have been removed will activate the alternative pathway, and the extent of activation is proportional to the amount of sialic acid removed. When the mechanism for this was investigated, it was discovered that, although C3b molecules on normal and desialated sheep erythrocytes demonstrated equal affinity for factor B, the affinity of C3b for factor H on fully sialated cells was 10- to 20-fold greater than on desialated cells (66). Thus, in this case, cell surface sialic acid, by increasing the binding of H to C3b, prevents the formation of an effective alternative pathway C3 convertase. A similar mechanism for control of alternative pathway activation was demonstrated for heparin that had been linked to zymosan particles. Interestingly, removal of sialic acid from human erythrocytes does not lead to alternative pathway activation. Thus additional control mechanisms are operative on human cells, some of which are discussed subsequently.

Role of Immunoglobulin

No obligate role for antibody exists in the activation of the alternative pathway, but immunoglobulin can exert a strong modulating influence on this system (67). Aggregated human myeloma proteins of the IgG, IgM, IgA, and IgE classes are capable of activating the alternative pathway (68). Soluble and particulate antigen–antibody complexes formed of IgG from several mammalian species and intrinsically nonactivating antigens have also been shown to activate the alternative pathway, and this can be shown to occur without classical pathway participation. Additionally, sensitization with specific IgG significantly augments the rate and/or extent of alternative pathway activation by intrinsically activating surfaces. This effect is independent of the Fc portion of the IgG molecule and has been observed to occur with both $F(ab')_2$ and Fab fragments.

The mechanisms whereby IgG influences the alternative pathway are several. IgG is an excellent acceptor of nascent C3b, the majority of which binds to a site in the heavy chain and probably within the Fd fragment (69). Thus antibody may enhance the deposition of C3b and the rate of formation of new C3 and C5 convertases by providing additional acceptor sites on a sensitized surface. Alternatively, antibody may function by masking sialic acid residues that would normally inhibit alternative pathway activation, as has been shown using antibodies to the capsule of group B streptococci (70). Finally, data suggest that C3b covalently bound to IgG is less susceptible to inactivation than free C3b or C3b bound to a non-immunoglobulin acceptor protein (71). This appears to be due to a reduced affinity of factor H for C3b bound to IgG. The relevance of this finding to surface-bound C3b remains to be established, but it may in part explain the observed relative resistance of C3b bound to IgG-bearing soluble antigen–antibody complexes to the action of factors H and I (72).

An interesting autoantibody has been shown to influence alternative pathway activation in a manner quite different from those previously discussed. This antibody, termed C3 nephritic factor (C3NeF), was discovered in the sera of patients with membranoproliferative hypocomplementemic glomerulonephritis and was subsequently shown to be present in some individuals with partial lipodystrophy (73). C3NeF is an IgG antibody specific for the alternative pathway C3 convertase, $\overline{C3bBb}$. The complex $\overline{C3bBb}$C3NeF is highly resistant to factor H-mediated dissociation and is thus capable of promoting unregulated C3 consumption by the alternative pathway.

Agents that Modify Alternative Pathway Activation

There are a number of substances that have proved to be useful modulators of alternative pathway activation in the laboratory. These can be divided into two groups, those that promote C3 cleavage and those that inhibit C3 cleavage.

One of the most widely used of such reagents is a 140,000-dalton protein from cobra venom that is functionally analogous to C3b. This three-chain molecule, termed cobra venom factor (CVF), is derived from the cobra's C3 (74). It can bind factor B, permit its cleavage by D, and form an alternative pathway C3 convertase. Unlike C3b, CVF is not inactivated by H and I and therefore forms an unregulatable C3-cleaving enzyme. The unregulated convertase is able to cleave C3 and factor B in the fluid phase until both proteins are essentially completely consumed from serum. This activity of CVF has been used by investigators to deplete C3 both *in vivo* and *in vitro* and as a way of obtaining C3b. The extent of consumption of terminal complement components following CVF activation of the alternative pathway depends on the source of CVF. Cobra venom factor isolated from the venom of *Naja naja kaoutha* forms not only an unregulated C3 convertase but also an effective C5 convertase. C5 cleavage is followed by consumption of all the terminal components. Cobra venom factor from *Naja haje* venom, on the other hand, is incapable of C5 cleavage; thus its use leads to consumption of B and C3 but leaves terminal component levels unaffected. The molecular mechanism for these differences in CVF molecules has not yet been elucidated.

Other agents that promote alternative pathway activation include suramin, a drug used in the treatment of African trypanosomiasis, and K-76 monocarboxylic acid, a product of the fungus *Stachybotrys complementi* K-76 (75,76). Both inhibit cleavage of C3b by factors H and I, albeit by different mechanisms.

Agents that inhibit alternative pathway activity include heparin and gold sodium thiomalate, both of which appear to increase the affinity of C3b for factor H (77,78). Fluid-phase heparin also appears to be capable of "masking" a portion of surface-bound C3b residues and preventing their interaction with factor B. Complestatin, a product of *Streptomyces lavendulae*, inhibits alternative pathway activation by combining with factor B in a manner that blocks its interaction with C3b. Recently, a macromolecular product of *Aspergillus fumigatus* has been identified which inhibits alternative pathway activation by as yet unclear mechanisms (79). In addition, a membrane glycoprotein encoded by herpes simplex type 1 virus has been shown to modulate both the alternative pathway C3 convertase and C3–C5 interactions (80). This may result in enhanced resistance of the virus to neutralization in serum (81).

THE C1 BYPASS PATHWAY

There is a third pathway of complement activation, discovered by the use of C4-deficient guinea pig serum. In this pathway, although no classical pathway C3 convertase is formed, C1 activation is required for activation of the complement cascade. Therefore this pathway has been called the C1 bypass pathway (82). Although the mechanism for this effect is unknown, one possibility is that activated C1 replaces D (albeit inefficiently) in concentrated C4-deficient serum. Initiation of complement activation via this pathway requires a large amount of antibody. The molecular natures of the C3 and C5 convertases in this pathway are unknown. Recently, this pathway has been shown to be responsible for the lysis of *Giardia lamblia* as well as several bacterial species (83).

TERMINAL COMPLEMENT COMPONENTS AND THE LYTIC MECHANISM

A detailed understanding of the molecular interactions of the terminal components C5 to C9 has developed over the last two decades. It is known that biologic activity of C5 requires cleavage of the C5 molecule into two fragments, C5a and C5b, by an enzyme derived from the C3 convertase. The C5a fragment has potent independent biologic activity which is considered later. The C5b portion combines sequentially with C6, C7, C8, and C9 to form a macromolecular complex that is capable of damaging biologic and artificial membranes and causing cell lysis by the creation of a hydrophilic transmembrane pore or channel (84,85). In addition, a phenomenon known as reactive lysis has been described, in which the isolated metastable C5b6 complex can be used to initiate formation of the terminal complex on surfaces without a concomitant requirement for earlier components. This reaction has provided a useful tool for identifying potentiators and inhibitors of terminal complex formation (86). The most intensive areas of investigation have focused on the exact molecular composition of the C5b-9 complex, the biochemical nature of attachment of this complex to membranes, and the functional and biochemical characteristics of the lytic complement lesion. After much debate, most workers in the field agree that complement lysis is due to the formation of a stable, hydrophilic transmembrane channel, as predicted by the "doughnut model" of Mayer et al. (85). There is now a general consensus that complement lysis of cells results because the terminal complex creates membrane channels that are large enough to permit exchange of small molecules and ions but too small to permit release of macromolecular cytoplasmic constituents. Due to the Donnan effect, water enters the cells through such channels causing the cell to swell and burst.

A large body of literature now substantiates that during formation of C5b-9, the complex inserts into the lipid bilayer of membranes. Evidence includes the observations that (a) hydrophobic domains are exposed within the forming C5b-9 complex, (b) phospholipids are released from target membranes during complement attack, (c) the attached C5b-9 complex cannot be eluted from membranes by ionic manipulations or aqueous-phase proteases but instead requires detergent for extraction, (d) conductivity changes are detected across planar lipid bilayers attacked by the terminal complex, and (e) constituents of C5b-9 can be labeled by membrane probes that localize exclusively within the hydrophobic core of lipid bilayers.

Initiation of C5b-9 Formation: C5 and the C5 Convertase

Initiation of membrane attack complex (MAC) formation requires cleavage of C5. C5 is a β-globulin glycopro-

tein comprising a 115,000-dalton α-chain disulfide linked to a 75,000-dalton β chain. Considerable sequence and structural similarity exists between C3, C4, and C5, but the latter lacks a thioester and does not form convalent bonds with target surfaces (51). Physiologic cleavage of C5 produces a 185,000-dalton C5b fragment and an 11,000-dalton C5a peptide. In cellular systems, the hemolytically active C5b remains cell associated whereas the smaller C5a fragment is released into the fluid phase. Recent data suggest that release of C5a is accelerated by interactions of C6 and C7 with newly cleaved C5 (87).

C5 is cleaved by enzymes formed during both alternative and classical pathway activation. The C5 convertase enzyme of the classical pathway is the $\overline{C4b2a3b}$ complex. Enzymatic activity resides in the $\overline{C2a}$ molecule within the complex. The enzymatic site for C5 cleavage by the alternative pathway convertase, $(\overline{C3b})_2\overline{Bb}$, is contained within $\overline{Bb}$. In both the classical and alternative pathway C3 convertases, physical decay of the enzymatic subunit from the complex results in loss of C5 convertase activity. Cleavage of C5 by either convertase results in identical fragmentation of the molecule. In each convertase, C3b binds native C5, exposing the site of enzymatic cleavage. Current data indicate that C3b covalently attached to C4b is the relevant C5-binding site in the classical pathway enzyme (88). Recent evidence suggests that there are two molecular forms of cell-bound C3b in the alternative pathway C5 convertase. One molecule appears to function like C4b in the classical pathway convertase, and one molecule is analogous to C3b of the classical pathway enzyme. The C3b serving as a C4b analog binds $\overline{Bb}$, maintaining it in an enzymatically active configuration. Fluid-phase C3b can also function as a binding site for C5, with subsequent cleavage by a fluid-phase enzyme complex. It is likely that the major requirement for effective cell surface C5 binding and cleavage is a high local concentration of C3b.

There is a linear relationship between the number of cell-bound C5 convertase complexes and subsequent C5 uptake. However, under conditions in which all C5 hemolytic activity is consumed, only a small percentage of C5 binds to the activating surface. Morever, cell-bound C5b rapidly loses hemolytic activity despite a substantially slower rate of loss of C5 antigen from the cell surface. Thus it appears that cell-bound C5b undergoes a rapid conformational change that renders it hemolytically inactive. The nature of the change is still unclear. As discussed in more detail later, the presence of C6 and C7 increases the uptake of C5 onto membrane surfaces and stabilizes the hemolytic activity of the cell-bound C5b.

Noncomplement-Mediated C5 Cleavage

A large number of noncomplement proteases produce cleavage of C5 to yield biologically active peptides. A detailed review of these reactions is beyond the scope of this chapter but has been well summarized (89). Briefly, trypsin, plasmin, polymorphonuclear leukocyte proteases such as elastase and cathepsin G, macrophage and platelet proteases, and bacterial enzymes have been shown to cleave native C5 into biologically active peptides. It is now clear that the fragments produced by trypsin cleavage of C5 are not equivalent to the fragments formed when C5 has been cleaved by a C5 convertase. Specifically, C5a is not produced, although a C5a-like biologic activity is generated. The anaphylatoxic and chemotactic activity of trypsin-cleaved C5 resides in fragments that remain disulfide linked to the high-MW, C5b-like polypeptide.

C5b6 and Reactive Lysis

C6, the next molecule in the cascade, is a β_2-globulin with a molecular weight reported between 105,000 and 128,000 daltons. Its structure is stabilized by numerous intrachain disulfide bonds (90). C5b and C6 can form a stable complex in serum that retains its ability to interact with cell membranes over time. With the addition of C7, C8, and C9, the C5b6 complex can lyse unsensitized erythrocytes and certain other cells in the absence of the antecedent components, a phenomenon known as reactive lysis (86). The conformational alterations in C5b which enable interactions with C6 are very short-lived, and C5 cleavage must occur in the presence of C6 for effective C5b6 complex formation to occur.

The biochemical characteristics of the C5b6 molecule have been defined (91). The complex has an S rate of 10.4 to 11.5, a MW of 328,000 to 330,000, and in its hemolytically active form contains a single molecule each of C5b and C6. The electrophoretic mobility of the isolated complex is indistinguishable from that of C5 (β_1) but faster than that of C6 (β_2). The C5b6 complex expresses a new antigenic determinant (neoantigen) that is not present on either C5 or C6 and is presumably related to conformational changes in the C5b and C6 molecules during complex formation (92).

C7, Formation of C5b67, and Inhibitors of C5b67

C7 has physical characteristics very similar to C6 and exhibits extremely high affinity for the C5b6 complex. It is reported to have 23 to 30% sequence homology with the later-acting components C8 and C9 (93). Attachment of C7 to newly formed C5b6 occurs very rapidly via hydrophobic interactions and results in the formation of a labile fluid-phase complex that aggregates and loses activity if not attached to acceptor surfaces. The C5b67 complex expresses a unique second neoantigen that differs from that of C5b6. This trimolecular complex represents the first product of complement activation to stably insert into the lipid bilayer of target membranes (94). The activity of the complex is very evanescent at physiologic temperatures and membrane insertion is inefficient (less than 1%) unless the target is sensitized with C3b. The presumptive mechanism of the C3b effect is binding of C5b to C3b, thereby promoting C5b67 formation close to the target surface and increasing the likelihood of subsequent hydrophobic interactions (95).

A number of substances have been defined that act on C5b67 reversibly in the fluid phase to prevent attachment to bystander erythrocytes during the short time that the hydrophobic binding site of C5b67 is available. One class of inhibitors includes the low-density lipoproteins and serum S protein, each of which presumably binds to the nascent hydrophobic membrane-binding site on C5b67 and blocks subsequent interaction of the complex with target membranes. A second class of inhibitors includes various polyanions such as heparin, dextran sulfate, and DNA. On the other hand, histones and polycations enhance the lytic activity of C5b67 for bystander erythrocytes. The mechanisms by which these inhibitors and potentiators act are still unclear, but presumably they function by modifying ionic or charge interactions of C5b6 or C5b67 with the target membrane. It has also been suggested that C8 limits the activity of C5b67 by preventing the attachment of the fluid-phase complexes to target membranes. Thus C8 serves a dual role in complement-mediated cytolysis and represents a point of internal regulation in the late steps of the complement attack sequence.

C8

C8 is a three-chain γ-globulin with α and β subunits of 64,000 daltons and a smaller γ chain of 22,000 daltons. The α and β chains differ in sequence despite their identical molecular weights (93,96). The α and γ chains form a disulfide-linked heterodimer which associates noncovalently with β chain in serum, where the complex exists as an equilibrium mixture. A single molecule of C8 can interact via its β chain with each C5b67 complex and this interaction has an extremely high affinity constant similar to that of C7 uptake by C5b6. When C8 binds to C5b67 on a membrane, the C8 γ chain is inserted into the hydrophobic regions of the phospholipid bilayer as assessed by labeling with hydrophobic photoreactive probes and inaccessibility to proteases. Insertion of C8 α chain through the lipid bilayer constitutes a lesion sufficient to initiate erythrocyte lysis, leakage of radiolabeled solutes, and significant increases in membrane conductivity (84). Target lysis by C5b-8 complexes is slow, however, and the hydrophilic lesions which are apparently formed are both small (0.4 to 3 nm) and unstable. Dramatic enhancement of lytic efficiency occurs on addition of the next component, C9.

C9

C9 is a single-chain α-globulin of 71,000 daltons. It is composed of two distinct domains. The smaller, C9a, is rich in acidic residues and hydrophilic. The larger domain of 37,000 daltons, C9b, is enriched for hydrophobic residues, although the deduced amino acid sequence does not include a single extended hydrophobic region similar to those of typical transmembrane proteins (94). Both C9a and C9b contain multiple intrachain disulfides which apparently do not cross domain boundaries. Antibodies elicited by denatured C9b are cross-reactive with C6, C7, and C8 α chain, as well as the pore-forming protein perforin, isolated from cytotoxic lymphocyte granules. Thus these proteins may have similar structures responsible for their hydrophobic interactions (94).

C9 has the capacity to polymerize, and purified C9 can be induced to do so by prolonged 37°C incubation. Polymerization is accompanied by elongation of the molecule, display of a distinctive neoantigen, an increase in surface hydrophobicity, and the appearance of free sulfhydryl groups. These latter participate in interchain disulfide bond formation, to produce C9 dimers. Polymerized (or "poly") C9 forms highly ordered tubular structures. These have a length of approximately 16 nm and an internal diameter of approximately 10 nm. One end bears a thickened annulus, while the other is hydrophobic and readily inserts into phospholipid bilayers. The average molecular weight of poly C9 is 1.1×10^6 daltons, but the complexes are heterogeneous and may contain 11 to 19 C9 monomers, although the more common forms contain 14 to 16 (84,94,97).

CHARACTERISTICS AND COMPOSITION OF THE FLUID-PHASE AND MEMBRANE-BOUND MEMBRANE ATTACK COMPLEX (MAC)

There is still controversy surrounding the details of complement lysis (84,94,97). In most situations the attack mechanism of complement requires the participation of C5b, C6, C7, C8, and C9 as a macromolecular complex with a molar composition of 1C5b:1C6:1C7:1C8:3–6C9. A stable fluid-phase 22.5S complex with a MW of 1.04×10^6 with the electrophoretic mobility of an α-globulin and containing C5b-9 can be demonstrated after incubation of serum with alternative or classical pathway activators. The complex assembled in the fluid phase has no activity but is able to inhibit lysis of EAC_{1-8} by C9, presumably because the fluid-phase complex, with a molar ratio of 1C5b:1C6:1C7:1C8:3C9, binds additional C9. Such complexes also contain three molecules of an additional protein, S protein (97). S protein circulates as a normal constituent of serum at a concentration of 600 μg/ml. This protein binds to the nascent hydrophobic binding site of C5b67 during formation of fluid-phase terminal component complex, thereby destroying its lytic activity and preventing its aggregation.

The physical characteristics of the membrane-bound MAC (C5b-9) have been studied following extraction from erythrocyte membranes by nonionic detergents. The complex within the membrane behaves like an intrinsic or integral membrane protein since it cannot be extracted with high ionic strength buffers or EDTA. This observation provided some of the first evidence that the MAC associated with the membrane through hydrophobic interactions. The dimensions of the extracted complex as determined by electron microscopy suggested the presence of monomeric C5b-9 complexes with an estimated MW of 1×10^6. However, others have since reported that the

membrane-bound form of the terminal complex is a C5b-9 dimer or consists of monomeric, dimeric, and trimeric forms, and the MAC may in fact have a heterogeneous size distribution.

Analysis of both cell-bound and fluid-phase C5b-9 complexes has suggested that they contain a disulfide-linked dimer of C9. Such C9 dimers may serve an important function in cell lysis by facilitating dimer formation of the entire C5b-9 complex, thus enhancing membrane disruption. However, this hypothesis remains to be proved.

Electron microscopic studies of the lytic complement lesion using the negative stain technique demonstrated that the lesion has the appearance of a doughnut, with an annular rim of 15 nm in diameter and a central, electron-dense region of 10 to 11 nm in diameter raised above the membrane (Fig. 4) (98). More detailed examination of the detergent-extracted complex from erythrocyte membranes has led to a model in which the complex contains a cylindrical stalk of 15 to 16 nm in length constituting the portion of the complex that is embedded into the hydrophobic core of the membrane. The annulus or torus of the complex projects above the membrane by at least 10 nm (Fig. 4), has an external diameter of 20 to 25 nm, and an internal diameter of 10 to 11 nm for human complement and 8.5 to 9.5 nm for guinea pig complement. When the isolated complex is reincorporated into lipid vesicles, the cylindrical axis of the complex is oriented perpendicularly to the membrane, and the annulus is located external to the vesicle (Fig. 4).

The interpretation of the electron microscopic appearance of the terminal complex has been modified in light of data developed by Podack and Tschopp (review in ref. 97). Based on the remarkable similarities between poly C9 and extracted MAC formed in C9 excess, these investigators have proposed that the membrane-bound MAC is composed largely of poly C9, with more limited participation of C5b678. Studies with membrane-restricted hydrophobic photoreactive probes are consistent with this view, since addition of C9 to membranes bearing C5b678 appears to reduce the exposure of the C5b678 to

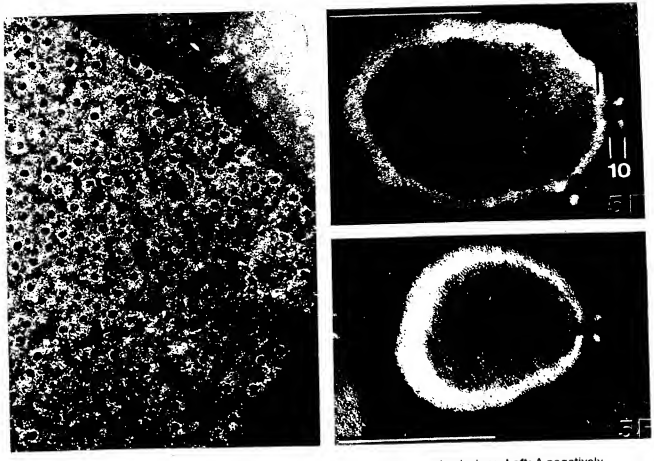

FIG. 4. Electron micrographs of the C5b-9 or membrane attack complex lesions. **Left:** A negatively stained erythrocyte membrane after complement attack, demonstrating innumerable discrete "holes." (Courtesy of Dr. R. Dourmashkin.) **Right:** A single complex penetrating a liposome. The long axis of the complex is perpendicular to the lipid surface and the hydrophobic domain is inserted 4 to 5 nm through the lipid milieu. The complex projects into the aqueous phase approximately 10 nm and displays a thickened outer annulus. (From Bhakdi and Tranum-Jensen, ref. 99, with permission.)

the hydrophobic milieu. C5b678 complexes in membranes bind C9 with extremely high affinity and with stoichiometries consistent with poly C9 formation (12–15C9:1C8). The resultant complexes appear to contain C5b and the C8 β chain in a rodlike 17-nm projection, while C6, C7, C8α-γ, and C9 are associated with the tubular lesion itself.

The formation of tubular poly C9 is not, however, a *sine qua non* for expression of the lytic properties of C5b-9 complexes. Functional transmembrane lesions are formed by complexes bearing numbers of C9 molecules well below that required for poly C9 formation, and the functional diameter of such channels is roughly proportional to the number of C9 molecules in the complexes (100). Furthermore, thrombin-cleaved C9, which cannot form poly C9, remains capable of supporting complement-mediated cytolysis. Thus while poly C9 formation may have an important role in the genesis of the classical MAC structure, the nature of the minimal effective lytic C5b-9 complex remains to be resolved.

CELLULAR COMPLEMENT RECEPTORS

Many of the biologic functions of complement are mediated by interaction of complement cleavage fragments with specific cell membrane receptors. The interaction of these complement component products with their receptors triggers a series of complex biochemical responses within the cells (101–103). Some of these fragments, such as C3b, iC3b, and C3dg, are bound to the site of complement activation on a target particle. The interactions of these target-bound components with their cellular receptors is thought to trigger a number of specific responses such as phagocytosis by neutrophils and macrophages, or B lymphocyte activation. Complement activation also generates several smaller peptides, C3a, C4a, and C5a, that are released into serum or extracellular fluid. This group of three polypeptides constitutes the complement-derived anaphylatoxins.

Anaphylatoxins and Their Receptors

The binding of these released polypeptides to specific cell surface receptors leads to a series of cellular responses important to the initiation and maintenance of the inflammatory process.

C3a is released on cleavage of C3 by either the classical or the alternative pathway C3 convertase. It is a 9,000-dalton, nonglycosylated protein with a pI of 9.7. C3a constitutes the N terminal 77 amino acids of the C3 α chain (104,105). Receptors for C3a have been identified on mast cells and basophils, smooth muscle cells, lymphocytes, and perhaps platelets. Engagement of the C3a receptors on mast cells or basophils induces degranulation, with release of histamine and other mediators of anaphylaxis; thus the name anaphylatoxin. Binding of C3a to tissue preparations induces contraction of smooth muscle cells. Whether smooth muscle contraction is mediated directly, or secondarily by histamine, is unclear. Although ileal contraction in response to C3a can be blocked by antihistamines, uterine contraction cannot (105,106). *In vitro* studies have also shown that C3a induces the secretion of mucus by goblet cells, another important feature of allergic and anaphylatoxic responses.

C5a, the "classic" complement anaphylatoxin, is an 11,000-dalton protein that represents the N terminal 74 amino acids of the C5 α chain. Approximately 25% of its MW is contributed by a single asparagine-linked oligosaccharide (104,105). Interestingly, the human peptide is heavily glycosylated but C5a from certain other species, such as the pig, has little or no carbohydrate. The function of the carbohydrate is unknown. In many systems removal of the carbohydrate leaves the activity of C5a unaltered. C5a is approximately 200-fold more potent as an anaphylatoxin than is C3a. Part of its anaphylatoxic effect is mediated by direct binding to a specific receptor on basophils and presumably mast cells. Part of its effect is indirect, mediated via binding to neutrophils which presumably then release a mast cell degranulation-inducing substance. C5a and C3a both show tachyphylaxis. Cells stimulated with one of these peptides will respond less well to a second stimulation with the same molecule. They will, however, respond to stimulation by the other anaphylatoxin. This is taken to indicate that each peptide binds to its own distinct and specific receptor. In addition to its anaphylatoxic effects on mast cells and smooth muscle, C5a also has histamine-independent effects on endothelium, causing increased vascular permeability. C5a also induces the directed locomotion (chemotaxis) of neutrophils and monocytes. The chemotactic receptor on these cells for C5a has been well studied (107). Binding of C5a to its receptor has a dissociation constant of 2 to 3 nM, and there are about 2×10^5 C5a receptors per neutrophil (108). In its ability to promote chemotaxis, C5a is quite different from C3a, which apparently has no effect on leukocyte chemotaxis. C5a has a number of other important effects upon neurotrophils, causing increased adhesiveness, aggregation, and adherence to endothelium, and also triggering both degranulation and oxidative burst activity (109).

C3a and C5a have also recently been shown to have important and opposite effects on *in vitro* immunoglobulin production by human lymphocytes. C3a suppresses and C5a enhances immunoglobulin secretion; both apparently act at the level of the T cell, presumably through receptor-mediated mechanisms.

A third peptide generated during complement activation, C4a, has only recently been shown to possess anaphylatoxic activity. It is a nonglycosylated, cationic peptide of 8,650 daltons. It is 100-fold less potent as an anaphylatoxin than C3a and 25,000-fold less potent than C5a. Its potential effects on neutrophils and lymphocytes have not been examined. Interestingly, C4a can elicit cross-tachyphylaxis to C3a, but not C5a, in guinea pig ileum. This suggests that C4a and C3a may act through the same receptor.

All the complement anaphylatoxins are rapidly cleaved in serum by the action of carboxypeptidase N, which removes the carboxy terminal arginine shared by all three molecules (104,105). The loss of the C terminal arginine

destroys the anaphylatoxic activity of all three molecules. However, $C5a_{des\ arg}$, although about 10-fold less potent than native C5a, does retain some chemotactic activity. A 60,000-dalton noncomplement serum protein called helper factor has been identified which, on binding to $C5a_{des\ arg}$, further increases its chemotactic activity without restoring anaphylatoxin activity (109). This protein may act to distort or obscure the carbohydrate present on $C5a_{des\ arg}$ since deglycosylated $C5a_{des\ arg}$ has been shown to be 10-fold better as a chemoattractant than unmodified $C5a_{des\ arg}$.

Integral Membrane Proteins that Regulate Complement Activity

One of the most important developments in complement research over the past 20 years has been the recognition of a series of integral membrane proteins present on cellular surfaces that regulate complement activation, degradation, and biologic activity. In many cases these proteins also function as membrane receptors. The binding of ligand to these receptors influences the activity or state of differentiation of the cells on which they reside.

The Receptors for Target-Bound Fragments of C3: CR1, CR2, CR3, and CR4

The best-studied cellular receptors for complement components are those involved in complement enhancement of the phagocytosis of bacteria, yeast, antibody-sensitized erythrocytes, and other particles. It has been shown that receptors for several C3 fragments are of great importance in phagocytosis. The discovery of cellular complement receptors is credited to Nelson in 1950. Although earlier reports are replete with examples of fresh serum promoting binding of particles to phagocytic cells, Nelson showed that neutrophil phagocytosis of *Treponema pallidum* and *Streptococcus pneumoniae* sensitized by antibody and complement was more efficient in the presence of human erythrocytes (110). This erythrocyte enhancement of phagocytosis was thought to occur because complement-coated bacteria were immobilized on the surface of the red cells and thus more easily engulfed. Ultimately, this red cell binding of bacteria was shown to occur because of the interaction of organism-bound C3b with a specific C3b receptor on the erythrocytes, a phenomenon known as immune adherence. All primate erythrocytes possess immune adherence (C3b) receptors; although nonprimate erythrocytes do not have this receptor, a functionally equivalent receptor exists on the platelets of nonprimate vertebrate species. Receptors for C3b have also been demonstrated to exist on B lymphocytes, some T lymphocytes, monocytes, and neutrophils as well as mast cells, eosinophils, basophils, and glomerular podocytes (102). The C3b receptors on all blood cells are antigenically identical but the receptor isolated from granulocytes has a slightly higher molecular weight on gel electrophoresis. The isolated C3b receptor glycoprotein is found in a series of genetically determined polymorphic forms and varies in size from 190 to 280 kd (see section on molecular biology). Its configuration in the cell membrane is unknown, although some evidence suggests that it exists in the form of a hexamer or pentamer. The C3b receptor, in order to distinguish it from membrane receptors for other C3 fragments, is termed CR1. There are 300 to 1,000 CR1 on each erythrocyte and about 5,000 to 30,000 CR1 per cell for the various leukocytes, as determined by binding of specific anti-receptor monoclonal antibody. Since there are about 1,000 times as many erythrocytes as leukocytes in the blood, this implies that over 90% of circulating CR1 are on erythrocytes. CR1 binds C3b with a much higher affinity than native C3, perhaps as much as 1,000-fold higher (63). Hence, the interaction of a C3b-coated particle or immune complex with the C3b receptor is not blocked by free serum C3, allowing C3b to play an important role in phagocytic processes and the catabolism of immune complexes. CR1 is freely diffusible in the membrane of all unactivated cells and when cells are extracted with nonionic detergents CR1 remains in the detergent-soluble fraction, suggesting that it is not associated with cytoskeletal elements. Presumably, in erythrocytes the molecule retains this configuration. On phagocytes, activation of the cell with lymphokines, phorbol esters, or a number of other soluble immunomodulators leads to clustering and ultimately capping of the receptor. It becomes detergent-insoluble, suggesting cytoskeletal attachment (111). Moreover, the receptor is endocytosed. Some data suggest that such endocytosis actually represents cycling of the receptor to an internal pool with subsequent return of the receptor to cell surface (112). If monomer ligand (C3b) is bound to the receptor, it too returns to the cell surface in unaltered form. However, internalized dimeric or multimeric C3b is transferred from the CR1-containing compartment to other compartments within the cell, where it is ultimately catabolized. Evidence obtained with phorbol ester-stimulated cells suggests that CR1 is rapidly phosphorylated and that phosphorylation is reversed after 30 min of incubation with the stimulant. Other stimuli, like the chemotactic peptide f-met-leu-phe, also cause phosphorylation although, unlike phorbol esters, they do not induce ingestion of targets such as C3b-coated red cells.

The binding of a C3b-coated target to CR1 does not itself initiate phagocytosis by a resting phagocyte. A second signal is generally required to initiate the phagocytic process (113). This signal can be provided by a few molecules of IgG bound to the target interacting with IgG Fc receptors on the cell membrane. It can also be provided by various activation signals that appear to take the cell from a resting state to an activated state. Such activation signals can be provided by factors released during chronic infection, fibronectin or laminin acting on mononuclear phagocytes, activators of the phosphoinositide metabolic pathway, and phorbol esters (103).

In addition to its role in phagocytosis, CR1 plays a critical role in the C3 degradative pathway. Upon binding of C3b to CR1, it becomes accessible to the action of factor I. In this respect it acts like fluid-phase factor H; however, the product of this interaction is not the fragment iC3b

mentioned earlier (see Fig. 3). In contrast, cleavage proceeds beyond the iC3b step to yield a further degradative product, C3dg (102,114). CR1 also facilitates the degradation of C4b by factor I, leading to the formation of a cleavage fragment, C4d, bound to the target surfaces. CR1 is believed to play a critical role in the processing of immune complexes. These appear to arise in the circulation quite commonly, for example, during the course of a viral infection, and are potentially quite toxic. It appears that the binding of C3b to these immune complexes is important in their ultimate removal from the circulation. Upon activation, C3b tends to bind to immunoglobulin (see previous discussion). Once immunoglobulin bound, the C3b tends to resist further degradation by factors H and I (71,114). The C3b will interact with adjacent cells with C3b receptors, and the bulk of CR1 receptors in the circulation are on erythrocytes. Such interaction leads to effective removal of the immune complexes from the plasma by adsorption to the erythrocyte surface, and the complexes can no longer diffuse from the intravascular compartment into tissue sites to induce damage. The C3b-containing immune complexes bound to erythrocytes circulate to the liver where they are stripped off the erythrocytes by macrophages within the hepatic sinusoid (115). The erythrocytes, now free of immune complexes, return to the circulation where they continue to circulate with a normal half-life. Interestingly, they appear to lose some membrane CR1 in this process, and states associated with the presence of high levels of circulating immune complexes are characterized by circulating erythrocytes with decreased numbers of CR1 (116).

CR3, CR4, and CD18 Family of Proteins

The product of C3b cleavage by factors H and I is a three-chain molecule, iC3b (see Fig. 3). There are receptors for iC3b on a variety of cell types including PMNs, monocytes, lymphocytes responsible for antibody-dependent and NK cellular cytotoxic activity, and mast cells (102,103). The iC3b receptor (termed CR3 or Mac-1) is functionally the best characterized of all the complement receptors. It is a two-chain molecule with a 150-kd α chain and a 95-kd β chain. This receptor is part of a family of such proteins, each with a distinct α chain, which all share the same β chain. Other members of this family (CD18) include LFA-1 (lymphocyte function antigen-1) and p 150-95, another C3-binding molecule now thought to represent CR4. The function of the β chain appears to be to direct insertion of the complex into the cell membrane (117). A portion of the cellular CR3 content resides within granules in neutrophils and is translocated to the cell surface when the cell is activated. The location of the receptor within the neutrophil has been reported by many workers to be the specific granule. However, a portion of the receptors may reside in another, more poorly defined, granule compartment that also contains the enzyme gelatinase. CR3 is quite specific in terms of ligand binding; in the presence of Ca^{2+} and Mg^{2+} ions, it will bind iC3b but does not recognize C3b. C3dg or C3d are bound weakly. It is reported that the receptor recognizes in part a characteristic arginine, glycine, asparagine (RGD) sequence in the α chain of C3, but that yet another binding site on the molecule is required for stable binding. The receptor is also reported to have conglutinin-like properties, binding to carbohydrate on C3 via a lectinlike interaction. This may represent the additional binding site (103). Like many receptors that recognize the RGD sequence, this receptor is reported to be important in cellular adherence and cells deficient in the receptor have a major adherence defect. A group of children have been reported who lack or have very low levels of the CD18 family of glycoproteins on their cell membranes. The phagocytes of these children ingest particulate targets poorly and also have multiple defects in other adherence-dependent functions. As a result of this deficiency, the children have frequent soft tissue and cutaneous infections with a variety of bacterial pathogens, especially staphylococci and *Pseudomonas aeruginosa* (118). Ligands that interact with proteins of the CD18 family are reported to appear on endothelial cells when these cells are treated with certain lymphokines, suggesting that the release of lymphokines facilitates the attachment of immune effector cells to endothelium, which is the first step in emigration into areas of tissue inflammation. In the phagocytic process itself, CR3 functions much like CR1, requiring a second signal for phagocytosis in the resting cell.

CR2 and Other Complement Peptide Receptors

This 140-kd integral membrane protein recognizes the physiologic C3 product formed upon interaction of CR1 with C3b and factor I, C3dg. Elastase and other tryptic enzymes can cleave C3dg to a further degradative fragment, C3d, which is also recognized by CR2. This integral membrane protein is present on all B lymphocytes and epithelial cells and is reported to be important in providing the B lymphocyte with signals that stimulate cell cycling and differentiation (119). CR2 also serves as the cellular target for the binding of Epstein–Barr virus to B lymphocytes. Its presence on epithelial cells is believed to be important in initial Epstein–Barr virus infections in which epithelial cells of the pharynx are first invaded. Although CR2 is not found on phagocytes, the C3dg or C3d fragments on a particulate target can facilitate binding and ingestion via interactions with CR3 and CR4.

There are receptors for several other complement components that have been identified on various cell types, but their biologic importance is not yet understood. A receptor for factor H has been reported on B lymphocytes and perhaps granulocytes and monocytes. Preliminary characterization of this receptor has shown it to migrate as a single 50,000-dalton band on SDS–PAGE analysis under reducing conditions (120). Binding of factor H to lymphocytes via this receptor is said to trigger factor I release from the cells, and exposure of monocytes to factor H increased NBT reduction and chemiluminescence. Lymphocytes, PMNs, and platelets also possess a receptor for C1q (121). There may be as many as 10^6 C1q re-

ceptors per neutrophil. The physiologic significance of this receptor is also unknown, but it is tempting to speculate that it may have a role in adherence of classical pathway activators to phagocytic cells. Since the C1 inhibitor binds to C1r and C1s and causes their release from macromolecular C1, C1q may remain attached to the classical pathway activator and be exposed to cellular receptors. Interaction of C1q with its receptor on monocytes and macrophages has also been shown to activate the cell to facilitate phagocytosis of both IgG-coated ligands and C3b/C4b-coated ligands. A macrophage receptor for Bb, which leads to macrophage spreading on glass or plastic surfaces, has also been reported.

Decay-Activating Factor (DAF), Homologous Restriction Factor (HRF), and Membrane Cofactor Protein (MCP)

These three membrane proteins do not act as typical receptors in that they do not promote the binding of a complement-coated target to the surface of cells expressing these molecules (although evidence is accruing that, in certain cases, cellular activation signals may be delivered by ligation of these glycoproteins). Nevertheless, these molecules do interact specifically with complement activation products and they play an essential role in preserving cellular integrity, preventing lysis of innocent bystander host cells resulting from spontaneous or induced complement activation.

Decay-accelerating factor (DAF) is a 70-kd membrane protein that is linked to the cell membrane by the diacylglycerol moiety of a phosphatidylinositol molecule which is covalently attached via a glycosidic linkage to the carboxy terminus of the protein (122). DAF inhibits formation of the classical pathway C4b2a convertase by interacting with C4b and preventing C2 binding, and also by destabilizing the convertase once it forms and enhancing its rate of decay. It also inhibits formation and promotes decay of the alternative pathway convertase, although it is somewhat less effective in this regard. DAF does not act as cofactor for cleavage of C3b or C4b (123). The molecule is present in the membrane of all blood cells and endothelial cells and exists in two forms, which differ in size and which are thought to result from alternative splicing of the terminal portion of the gene encoding DAF (124). One form of the protein is processed to provide the phosphoinositide membrane linkage and the other is truncated and released from the cell without the membrane anchor moiety. It is also reported that the form of this protein found in neutrophils is slightly heavier than that found in erythrocytes. Like HRF, this phospholipid-anchored membrane-protective molecule is missing from the cells in patients with the disease paroxysmal nocturnal hemoglobinuria (PNH) (125,126). These abnormalities appear to account for at least a portion of the exquisite complement sensitivity of erythrocytes in PNH and are likely responsible for chronic intravascular hemolysis in this disease.

Homologous restriction factor is a 65-kd regulatory protein which is also phospholipid anchored and has been studied thus far on lymphocytes and erythrocytes, although it has been shown to be present on monocytes, neutrophils, and platelets as well (127,128). It interacts with both C8 and C9 of the membrane attack complex to prevent successful insertion of the complex through the membrane bilayer, thus protecting cells from the late-acting steps in complement attack. It derives its unusual name from the observation that, in the case of each species thus far studied, the factor recognizes and interacts with homologous C8 and C9 far better than heterologous C8 and C9. The presence of this factor in cell membranes would appear to explain the fact that complement proteins are far more effective at lysing cells of heterologous species than they are at lysing cells of the homologous species. HRF is also reported to inhibit the action of the cellular cytotoxin termed perforin or cytolysin, a molecule found in large granular lymphocytes which has significant homology to C9 and which has been reported to cause cell lysis (129).

Yet another membrane protein termed membrane cofactor protein (MCP) or gp45-70 has been reported in the membranes of most blood cell types, but not erythrocytes. In addition, it is reported to be present on epithelial cells, fibroblasts, and endothelial cells. This protein binds to C3b and iC3b but does not appear to have sufficient affinity to act as a complement receptor per se. It does, however, facilitate the factor I-dependent degradation of C3b to iC3b and may in fact be more efficient in this regard than any of the previously identified fluid-phase or membrane cofactors (130). Interestingly, it does not have decay-accelerating activity for either the C3 or C5 convertases.

MOLECULAR BIOLOGY, SYNTHESIS, AND DEFICIENCIES OF COMPLEMENT PROTEINS

Within the past decade the molecular biology of the complement system has come under intense scrutiny. DNA encoding nearly every component of the cascade, as well as regulatory factors and cellular receptors, has been cloned and sequenced. Chromosomal mapping of the complement genes and studies of the regulation of synthesis of several components have been performed with molecular probes. An encyclopedic review of these data is beyond the scope of this chapter and is available elsewhere (131). We treat several major themes which have emerged from these studies.

The C3b/C4b Binding Protein Superfamily

A large number of plasma and membrane proteins interact with C3b or C4b. These proteins are important in complement activation, regulation of the cascade, or as cellular receptors. They include factor H, C4BP, CR1, CR2, DAF, gp45-70 (MCP), C2, and factor B (132,133). A close relationship between these proteins was first suggested for the regulatory plasma proteins H and C4BP,

and the regulatory and membrane receptor protein CR1, on the basis of classical genetic techniques. By studies of electrophoretic polymorphic variants, the genes for H, CR1, and C4BP were found to be very closely linked in the human. As molecular probes and eventually full-length cDNA clones for these proteins have become available, a gene superfamily has emerged. At least the CR1, CR2, C4BP, H, and DAF genes are located on the long arm of chromosome one in the human (134). More importantly, each member of this group has been shown to contain from 8 to over 30 short homologous repeating units approximately 60 amino acids in length. These repeating units generally appear in sequence and occupy the amino terminal portion of the protein (133). There are eight or nine highly characteristic conserved amino acids, including four cysteines, as well as conserved hydrophobic regions. The deduced structure of CR1 has shown its 28 to 33 short consensus repeats to be organized into longer homologous repeats, each of which contains seven of the shorter segments. Duplication en bloc of one of these longer homologous repeating segments is thought to have given rise to at least one of the more common electrophoretic variants (103,131). The deduced structure of CR2 appears similar. The CR1, CR2, C4BP, and DAF genes are very closely spaced and share a higher degree of homology, while factor H is not as closely linked and is also more dissimilar in primary sequence (134). The C3b/C4b binding capacity and cofactor activity of these proteins have been explicitly shown to reside in the repeating units in the case of H and C4BP, and this relationship is presumed to hold true for the remainder of the family. While less well studied, the organization and structure of the murine C3b/C4b binding protein genes appear similar.

C2 and factor B, which also bind and interact with C4b or C3b, each contain three of the typical short consensus repeats at their amino termini (133,135). The carboxy terminal ends of these proteins confer serine protease activity. C2 and factor B are encoded on chromosome 6 in the human, within the major histocompatibility complex (MHC), and are discussed further later. Two other complement components, C1r and C1s, have also been found to have two of the homologous repeating units at the carboxy terminal end of their noncatalytic heavy chains. Several noncomplement proteins: β_2-glycoprotein 1, factor XIII, and the (p55 chain of) IL-2 receptor also contain several copies of the short homologous repeating unit. Careful studies of genomic clones for several proteins in this superfamily indicate that each short repeat may be encoded by a discrete exon. The functional significance of these structures and the factors underlying the presumptive multiple gene duplications responsible for this family remain to be determined.

The MHC-Linked Complement Genes

The genes encoding C2, factor B, and C4 lie in a 120-kb stretch of DNA between the HLA-DR and HLA-B loci on chromosome 6 in the human (131) (Fig. 5). The C2 and factor B genes are less than 1 kb apart. These two genes demonstrate high primary sequence homology and each encodes several amino terminal short homologous repeats linked to a carboxy terminal serine protease domain as described previously. The exon structure of the DNA encoding the protease domain of factor B and C2 is similar, but not identical, to that of other serine proteases. The C2 and factor B genes contain a unique exon encoding a region of polypeptide chain between the Asp and Ser residues of the active site that is not found in other serine proteases and share an overall 33% homology in their enzymatic domains. Thus C2 and factor B almost certainly represent a gene duplication (135).

Approximately 30 kb distant from the C2 and factor B genes lie two separate C4 loci, each associated with a gene encoding the noncomplement protein steroid 21-hydroxylase. The two C4 genes encode the two isotypic variants C4A and C4B. Fragments of C4A and C4B deposited on red cells are responsible for the HLA-linked Rodgers and Chido blood group antigens, respectively. The homology between C4A and C4B is approximately 99%, with perhaps as few as six amino acid substitutions defining the two isotypes. These isotypic variations are clustered in the portion of the C4 molecule which gives rise to the C4d fragment and contains the reactive thioester site (136). The functional consequence of the substitutions appears to be a predilection of C4A to form amide bonds with proteins, whereas C4B preferentially forms ester bonds with carbohydrate moieties. In studies of erythrocyte lysis, C4B is far more active than C4A. Multiple allotypic variants of each C4 isotype exist, so that a normal com-

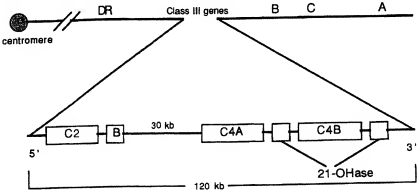

FIG. 5. A map of the MHC-linked complement genes (class III MHC gene products) on the short arm of chromosome 6 in the human. The arrangement of the HLA, A, B, C, and DR loci is shown relative to the complement genes. The expanded 120-kb segment shows the relative position and size of the C2, factor B, C4A and B, and 21-hydroxylase (21-OHase) genes. Not drawn strictly to scale.

plement of C4 genes may encode up to four distinct forms of the protein. Furthermore, null alleles at both loci are fairly common, and 10 to 15% of the normal population may carry at least one null (or Q0) C4 allele. About half of null alleles thus far examined result from large deletions which involve most or all of one C4 gene and an adjacent 21-hydroxylase gene. While less well documented, evidence is also accruing that a significant number of haplotypes in the general population may contain three C4 loci. The high frequency of both large deletions and apparent duplications has led to the proposal that unequal crossover events between the C4 loci of sister chromatids during meiosis may be responsible for such haplotypes with one or three C4 genes (137). In the mouse, only one functional C4 product is found. A second C4-like protein is encoded in the major histocompatibility complex, and expression of this protein is under the control of sex hormones (sex-limited protein or SLP). Absence of C4 function in SLP is probably related to accumulated sequence changes near the $\overline{C1s}$ cleavage site (131).

Because of their proximity in the genome, specific combinations of C2, factor B, and C4 allelic variants tend to occur together. Crossovers within these combinations are uncommon, and they tend to be inherited as units referred to as complotypes (131). Complotypes can serve as markers for even larger units which are called "extended haplotypes" and include the HLA A, B, C, and DR loci. These are usually inherited en bloc and occur in the general population at frequencies higher than those predicted by their physical proximity alone. It has been suggested that crossovers within extended haplotypes are actively suppressed, but the mechanisms and selective pressures responsible for this remain speculative.

Biosynthesis of Complement Components

Over the last several decades there has been a steady accumulation of data relating to the biosynthesis of complement components. In the human, the liver appears to play a preeminent role in the synthesis of most of the components. This has been shown clearly for C3, C6, C8, and factor B by documenting a switch in the circulating allotypes of these proteins to those characteristic of the donor after orthotopic liver transplant (138). Notably, the recipient's complement component allotypes do not disappear completely after such surgery, suggesting extrahepatic synthesis as well. Data regarding the site of synthesis of most other components are derived primarily from *in vitro* studies of primary tissue cultures or established cell lines, both animal and human. On the basis of such information, it appears that functional C1 macromolecules are produced by a variety of epithelial cells of endodermal origin, and these cells, as well as macrophages, are thought to be an important locus of *in vivo* production (139). The source of virtually all the other components studied appear to be the liver or the mononuclear phagocyte system or both (140). While mononuclear phagocytes probably contribute little to total plasma complement levels, their output may be of considerable importance at local sites of inflammation.

Regulation of complement protein biosynthesis is complex. The majority of studies have been carried out with primary macrophage cultures and hepatocyte or macrophage-like cell lines. Synthesis is responsive to a wide variety of modulators including immune complexes, ambient complement protein levels, cytokines, neuropeptides, histamine, and arachidonate metabolites, and the responses evoked are both protein and tissue specific (140). Regulatory mechanisms have been demonstrated at the pretranslational, translational, and post-translational levels in various *in vitro* systems. At the clinical level, the majority of complement proteins are elevated in plasma during acute-phase responses. Careful studies of regulation of C2 and factor B synthesis using molecular probes are in progress. Despite divergent, independent responses of the synthesis of these two proteins to a variety of stimuli, it appears that at least one segment of DNA 3′ to the C2 coding sequence is essential for expression of both genes (141). It is interesting to note that at least two complement proteins, C1 and C8, are composed of two or more gene products which are assembled noncovalently in the extracellular compartment after secretion. The plasma pool of these components exists as an equilibrium mixture of complexed and disassembled subcomponents, and synthesis of the various subcomponents is clearly separable in both *in vitro* systems and genetically deficient individuals.

Complement Deficiency States

Complement deficiency states are distinctly uncommon, and this low frequency points to the strong selective pressure favoring maintenance of an effective complement cascade. Complement protein alleles, including null alleles, show codominant behavior. Thus heterozygotes for null alleles have roughly half-normal levels of the components in question and are usually clinically normal (with one exception—see later discussion). The consequences of homozygous deficiency states divide roughly in accordance with the portion of the cascade involved. Humans with deficiencies of the alternative pathway are highly susceptible to a variety of pyogenic bacterial infections (presumably due to failure of C3-dependent opsonization), whereas those with terminal component defects have a poorly understood isolated propensity to disseminated neisserial infections. A substantial proportion of individuals with terminal component deficiencies, especially C9, are, however, entirely well. Autoimmune disease, presenting as glomerulonephritis or systemic lupus erythematosus-like syndromes, is seen in deficiencies affecting all three portions of the complement cascade but is the particular hallmark of classical pathway defects (142,143). Such disorders are seen in over half of individuals with C2 or C4 deficiency. The genesis of this striking relationship is unclear. It has been proposed that C2 and C4 deficiencies profoundly alter the disposition of immune complexes and thereby promote both end-organ pathology and persistent immunoregulatory abnormalities (144). It is notable, however, that heterozygous family

members of C2-deficient patients and humans whose genotype includes even one null allele (especially C4AQ0) among the four C4 genes have increased incidences of autoimmunity (142). This finding, and the relationship of C2 and C4 genes to the MHC loci, has raised the question of additional disease-susceptibility genes linked to the null complement alleles. These questions have not been resolved.

Deficiency of C1 inhibitor in the human is clinically manifest in heterozygotes as the syndrome of hereditary angioedema (145). Affected individuals are subject to recurrent localized soft-tissue swelling. C1 inhibitor is an important regulator of the clotting, kinin-generating, and fibrinolytic enzyme systems in addition to the complement cascade. Chronic consumption of the inhibitor by these enzyme systems outstrips the output directed by one normal gene, and C1-inhibitor titers fall well below half-normal levels. Failure of homeostatic regulation of one or more of the above protease systems is then believed to generate a vasoactive mediator(s) which results in angioedema. The precise mediator has been extensively sought but remains uncertain.

A number of animal models of complement deficiency are available. These include C2-, C3-, and C4-deficient guinea pigs, C3-deficient dogs, C5-deficient mice, and C6-deficient rabbits (146). These have been useful in elucidating the role of complement in opsonization and in confirming abnormalities of the immune response to prototypic antigens. Some of these animals exhibit clinical diseases or subclinical serologic abnormalities that mimic their human counterparts.

CONCLUSION

As recently as 1969, Macfarlane Burnet wrote in his book *Cellular Immunology*:

> Since the studies of Bordet, Ehrlich and Wasserman, the concept of complement as an essential part of the mechanism of immunity has progressively been replaced by a rather uncertain decision that the classic phenomenon of complement lysis of red cells is a laboratory artifact of no real significance for immunity.

We hope that it is clear that our understanding of complement has come a long way since that time. We are now certain that the complement proteins play a critical role in host defense and the development of autoimmunity. We believe it likely that these proteins will prove to be important in control of a number of steps in the immune response as well. The fact that very few individuals are missing any of the many proteins and the fact that the proteins show remarkable evolutionary stability suggest that their further study will reveal new important control functions. Now that the chain structure of most of the proteins and cleavage fragments is known and the amino acid sequence of many of the proteins is established, understanding their interactions and the biologic consequences of their activation will provide one of the major challenges of the next decade.

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